Karolinska Institutet

Department of Physiology and Pharmacology

INORGANIC AND ORGANIC NITRATES AS SOURCES OF NITRIC OXIDE

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Farmakologens föreläsningssal, Nanna Svartz väg 2, Karolinska Institutet

Fredagen den 8 juni, 2012, kl 09.00

av

Mirco Govoni

Huvudhandledare:
Professor Jon Lundberg
Karolinska Institutet
Institutionen för Fysiologi och Farmakologi

Bihandledare:
Professor Loris Grossi
University of Bologna, Italy
Department of Organic Chemistry

Fakultetsopponent:
Professor Paul Winyard
University of Exeter, UK
Peninsula College of Medicine and Dentistry

Betygsnämnd:
Docent Fredrik Palm
Uppsala Universitet
Institutionen för Medicinsk Cellbiologi

Professor Jesper Häggström
Karolinska Institutet
Institutionen för Medicinsk Biokemi och Biofysik

Professor Göran Engberg
Karolinska Institutet
Institutionen för Fysiologi och Farmakologi

Stockholm 2012

ABSTRACT

Inorganic nitrate (NO3-) and nitrite (NO2-) have generally been considered stable inactive end products of nitric oxide (NO) or unwanted residues in the food chain. While several recent studies surprisingly demonstrate that nitrite is reduced to bioactive NO in blood and tissues, the more stable anion nitrate is still considered to be inert.

We investigated if inorganic nitrate could be bioactivated in vivo to generate nitrite systemically. After oral intake and absorption, nitrate is concentrated in saliva, where much is reduced to nitrite by oral bacterial nitrate reductases. We show here that systemic nitrite levels increase greatly after oral nitrate intake, demonstrating for the first time that nitrate is in fact a substrate for systemic generation of nitrite and eventually NO. We show that oral bacteria and the entero-salivary recirculation of nitrate play a major role in the in vivo bioactivation of nitrate. In addition to this major prokaryotic pathway, we discovered a mammalian functional nitrate reductase (eukaryotic pathway) that also regulates nitrite and NO homeostasis.

Subsequent studies have confirmed robust physiological effects of dietary nitrate, all of which are compatible with generation of NO. These include a lowering of blood pressure and inhibition of oxygen consumption in humans, and protection against ischaemia-reperfusion injury and reversal of metabolic syndrome in animals. This has made us speculate that the strong cardioprotective effects of a diet rich in vegetables, at least partly is explained by the high nitrate content in these foodstuffs.

Differently from inorganic nitrate, organic nitrates such as glyceryl trinitrate (GTN) are generally recognized to act via NO donation, and these drugs have been used in the treatment of cardiovascular disorders for >100 years. Despite this long history, their metabolism is still a matter of debate. It is known that liver first pass metabolism can strongly affect their disposition and activity. Thus a careful investigation of the hepatic metabolism is crucial for compounds designed for oral administration. In the second part of this project the liver metabolism of a novel class of hybrid organic nitrates, the nitrooxybutyl-esters derivatives of anti-inflammatory or anti-oxidant compounds was investigated and compared with the prototypic organic nitrate GTN. These compounds are claimed to retain the properties of the parent compound with increased, NO-related, safety and tolerability. It was shown that nitrooxybutyl-ester derivatives are rapidly cleaved in vitro in liver fractions to their parent compounds and the organic nitrate moiety nitrooxybutyl alcohol (NOBA). As for GTN, NOBA is mainly denitrated by the glutathione S-transferase through a clearance based mechanism, i.e. direct metabolism to NOx (nitrite + nitrate) with no main acute bioactivation to NO. The NOx generated during first passage could therefore contribute to the “NO related” effect of organic nitrates when given orally. Moreover, since NOBA is only slowly denitrated in the liver in vitro it might have the potential to partly survive first passage metabolism and be bioactivated to NO in other tissues. A complete picture of the metabolic profile of this class of organic nitrates in different tissues will help to facilitate development of more powerful and selective drugs in different therapeutic areas.

In conclusion the results of the present thesis laid the bases that reversed the status of inorganic nitrate from inert end product of NO metabolism to important reservoir of NO. It follows that also the nitrate and nitrite generated from organic nitrate metabolism might play an important role in the final biological effect of these molecules.

ISBN 978-91-7457-635-1